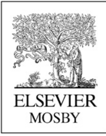

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ADVANCES IN OPHTHALMOLOGY AND OPTOMETRY

Polymerase Chain Reaction in the Diagnosis of Uveitis

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Keywords

• Polymerase chain reaction • PCR • Uveitis • Inflammatory eye disease • Infectious eye disease • Noninfectious eye disease

Key points

- Polymerase chain reaction (PCR) has improved diagnostic capabilities in infectious uveitis, especially in atypical clinical presentations of herpes viruses and toxoplasmosis.
- PCR has improved diagnostic capabilities in noninfectious causes of uveitis, specifically primary vitreoretinal lymphoma, allowing patients to receive proper treatment in a timely manner.
- PCR has been proven to be a highly sensitive and specific diagnostic tool that is widely available in modern research and clinical laboratories.

INTRODUCTION

The polymerase chain reaction (PCR) allows scientists to amplify a known sequence of DNA and use the amplified fragments to detect the presence or absence of the DNA product in a given sample. Since the advent of the PCR in the late 1980s, the technique has been altered and is now known to take many forms, including the multiplex PCR, real-time PCR, and broad-range real-time PCR, to name a few. In the ophthalmology clinical practice and research realm, this tool is used to diagnose viral, bacterial, fungal, and parasitic causes of infectious uveitis, as well as noninfectious masquerade uveitides such as intraocular lymphomas. In comparison with other diagnostic methods, PCR may be easier to perform and require a smaller sample of ocular fluid and

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less time to realize results. However, a small amount of contamination may have a significant impact on results, and resource-poor areas of the world may have difficulty accessing the necessary equipment and environment required. The use of PCR in clinical practice would likely increase if the list of identifiable pathogens expanded, cost was reduced, and the tools required to perform this experiment became more accessible to less developed health care systems.

THE POLYMERASE CHAIN REACTION

A brief history

The advent of the PCR in the late 1980s has proven to be among the most substantial advances in science of the twentieth century. This development has allowed scientists to analyze a known nucleotide sequence both qualitatively and quantitatively. First published in 1985, K.B Mullis and F. Faloona [1] developed the procedure to characterize mutated alleles of the β-globin gene, which were known to be associated with sickle cell anemia. Today, PCR continues to be used for similar purposes but its use has expanded to include detection of tumor markers in patients with cancer [2], identification of perpetrators at the scene of a crime [3], and detection of infectious and noninfectious agents. Before the advent of PCR, scientists found it difficult to characterize DNA because either large amounts of extraneous material were present or not enough DNA was available in a given sample to yield significant results. The reagents used to perform the reaction help to minimize the former problem, whereas the PCR itself solves the latter.

What is required for the reaction and how it is performed

The basis of PCR is to amplify a known region of DNA or RNA. Reverse-transcriptase PCR (RT-PCR) is used to identify RNA viruses and first involves transforming an isolated RNA sample into copy DNA via reverse transcriptase. After this initial step, the process for amplification of DNA and RNA is identical. The PCR is conducted via combining a DNA sample (which serves as the template to be amplified), 2 DNA primers, a heat-stable DNA polymerase, a buffer solution, and deoxynucleotide triphosphates (dNTPs) in a 0.2 mL tube. The tube is then placed in a thermal cycler, which allows for the reaction to occur in 3 steps: denaturation, annealing, and elongation (Fig. 1) [4]. The denaturation step heats the sample to roughly 95°C, thus causing the double-stranded DNA sample to break, resulting in 2 single strands. The annealing step is performed around 60°C and allows the DNA primers to bind to the single-stranded DNA segments now in solution. Elongation occurs at roughly 80°C. At this stage, the heat-stable DNA polymerase synthesizes complementary strands of DNA using the bound DNA primers as a starting point and the dNTPs as the bases for the new DNA segment. The result of denaturation, annealing, and elongation is 2 double-stranded DNA segments identical to the region between the DNA primers. The 3-step PCR reaction is repeated 30 plus or minus 10 times to yield a large copy number of the known

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